Blueberry yields heightened in response to *Clonostachys* spray treatments

John Sutton*, Bradford Rooney** and Todd Mason***

The microscopic fungus *Clonostachys rosea* is currently being developed for farm use as a tool to promote the health and productivity of many kinds of crops. In nature, the fungus occurs within the leaves, stems, flowers, fruits and roots of a wide diversity of plants in temperate and tropical climates around the world. Over several years, particular strains of the fungus have been selected and tested in crop plants for activity against diseases and for promoting growth, vigour and yields. Recent work in small field plots and the laboratory has shown that our strains of *Clonostachys* are able to strongly suppress most economically important diseases of lowbush blueberries encountered in Canada including Monilinia (mummyberry), Phomopsis canker, Sclerotinia berry drop, and Botrytis. In 2014 we conducted trials to determine the effectiveness of *Clonostachys* as a practical tool for promoting the health and productivity of blueberries when applied under actual farm conditions. In particular it was important to determine when *Clonostachys* treatments should be applied during the crop season so as to achieve the best effects against disease outbreaks and in terms of better berry yield and quality.

The trials were done in relatively large plots (e.g. 0.5 – 0.9 acres) in blueberry fields in Prince Edward Island using standard farm equipment including a tractor drawn sprayer and a mechanical ride-on harvester. Spray applications were timed during the growing season based on protocols derived from our earlier work and from knowledge of how the diseases develop in relation to crop growth and weather conditions. The different treatments involved sprays at the V1 leaf stage, the V4 leaf stage, 20% bloom, and combinations of these; untreated plots served as controls. It should be recognized that the growth stages at the time of spraying were approximate especially because of variability among the clones present in the trial areas. Simple use protocols were implemented also for handling and applying the *Clonostachys* product and for timing each spray in relation to weather conditions during and after spray application.

All sprays employed a standardized rate of 1 gram formulated powder per litre of water plus surfactant (1.5 L soybean oil / 1000 L), which was equivalent to 200,000 to 300,000 spores of *Clonostachys* per millilitre of spray material. Sprays were applied at 156 L / acre. The V1 sprays were applied at 6 p.m. AST on 17 May (air temperature 15°C, declining to 8°C within 12 hours); the V4 sprays were applied at 6 p.m. on 29 May (air temperature 22°C, declining to 10°C within 6 hours and to 8°C within 12 hours); the bloom sprays were applied at 9 a.m. at site 2 (Ruel's B) on 3 June (air temperature 20°C decreasing to 13°C with fine mist during 8 hours), and at 9 a.m. at site 1 (Homefarm H) on 4 June (air temperature 17°C increasing to 22°C within 8 hours, with no precipitation).

CLONOSTACHYS SPRAY TREATMENTS INCREASED BERRY YIELDS BY 46-116% (Table 1).

The *Clonostachys* treatments increased berry yields in the range of 46% to 116%. The greatest increases were observed for treatment 2 in which two sprays were applied respectively on 29 May (stage V4) and 3 or 4 June (20% bloom). A single application at

20 % bloom (treatment 3) gave a 68% yield increase at site #2. The spray applied at the V1 in treatment 1 is thought to have been ineffective in part because temperatures were not sufficiently high for *Clonostachys* to establish well in the foliage.

Table 1. Effects of timing of *Clonostachys* spray applications on estimated berry yields at two trial sites.

Treatment number	Spray timing	Site #1 (Homefarm H) Yield lbs/acre	Site #1 (Homefarm H) Yield % increase *	Site #2 (Ruel's B) Yield lbs/acre	Site #2 (Ruel's B) Yield % increase *
1	V1, V4, 20 % bloom	2,194	66.4%	1,491	45.7%
2	V4, 20% bloom	2,477	87.8%	2,211	116.1%
3	20% bloom	1,347	2.1%	1,725	68.6%
4	None (control)	1,319	--	1,023	--

*compared to the control

Yield data were collected also in an additional field (O'Connors) which was sprayed once with *Clonostachys* at the 20% bloom stage. This spray was applied on a night (3 June, 11:00 p.m.) when leaf wetness (8 hours) was prolonged in association with misty rain, which favoured very effective establishment of *Clonostachys* in the flowers and foliage (see below). In this case berry yields were measured in five separate areas (each 9 square feet) in each of four zones within the field (Table 2).

Table 2. Berry yields in a field (O'Connors) treated with a *Clonostachys* spray at 20% bloom.

Sampling zone	Range of berry yields among 5 samples (lbs/acre)*	Mean yield for zone (lbs/acre)*
A	936 - 2498	1,452
B	455 - 8475	4,432
C	312 - 3836	2,116
D	820 - 7824	3,684
	MEAN YIELD (all samples)	**2,917**

*Each of the five berry samples of each sampling zone were taken by hand rake from a crop area of 9 square feet, weighed, and the values were adjusted to lbs / acre.

The mean berry yield of 2,917 lbs / acre in the O'Connors field (Table 2) was high in comparison to the untreated controls and *Clonostachys* treatments in the spray trail (Table 1). It is clear from the results (Table 2) that berry yields were extremely variable within and among the sampling zones. In our experience this variability is the norm for

blueberry crops grown in this and many other regions on account of factors such as relatively high clone diversity, crop patchiness, weed patches, differences in available soil nutrients, localized soil compaction, and microclimatic conditions. Substantial confidence can be placed in the value obtained for the estimated mean yield given that it is based on a total of twenty samples. Use of larger strip plots such as employed in the trials reported in Table 1 help to minimize confounding effects of the different variables.

CLONOSTACHYS ESTABLISHED EFFECTIVELY IN THE BLUEBERRY FOLIAGE AND FLOWERS FOLLOWING SPRAY APPLICATIONS.

The beneficial effects of *Clonostachys* on plant health and productivity depend heavily on the ability of the fungus to establish *inside* the plant tissues. For blueberries it is important that spores of the fungus applied in foliar sprays grow and establish in the leaves and flowers since these are key portals through which pathogens invade the crop, including those that cause mummyberry, Phomopsis, and Sclerotinia berry drop. The spores of *Clonostachys* require several hours of humid or wet conditions at favourable temperatures (e.g. 12 - 20°C) in order to germinate and penetrate into the plant tissues. Note that plants colonized by *Clonostachys* do not show any kinds of symptoms except that the leaves may sometimes appear a bit greener.

Sprigs of treated and untreated blueberries were sampled in the two trial sites following each spray application and analysed in the lab to determine the % incidence of leaves and flowers that were colonized by *Clonostachys*. **In general, establishment of *Clonostachys* in the crop was very good following the second (V4) and third (20% bloom) sprays but weak following the first (V1) spray.** Establishment was low (6-12 % of leaves and flower buds) following the first spray (V1 stage) largely because temperatures were low, but high (60-80%) following the second spray (V4 stage) when temperatures were several degrees warmer and moisture conditions also favorable. *Clonostachys* established in over 60% of the opened flowers following the 20 % bloom spray at site #2 (Ruel's B) where conditions after spraying were very favourable for the fungus, but at only 20-30% of flowers at site #1 (Homefarm H) where conditions were somewhat dry.

CLONOSTACHYS SPRAY TREATMENTS MARKEDLY REDUCED THE SEVERITY OF THE ECONOMICALLY IMPORTANT DISEASES

Diseases were assessed in sprig samples of treated and untreated blueberries taken at intervals (27 May, 9 June, and 8 July) during the growing season. The main commercially important diseases present in the field trials were **mummyberry, Phomopsis and Sclerotinia berry drop. *Clonostachys* substantially reduced the severity of each of the three diseases when applied a few days before bloom (V4) *and* at early (20%) bloom OR at early (20%) bloom *only.***

Mummyberrywas not found in samples of 27 May but was present in samples of all treatments taken on 9 June. However, in later samples taken on 8 July mummyberry was much milder in sprigs that received both the V4 and bloom sprays compared to the untreated controls, and substantially milder compared to plants sprayed only at bloom.

Phomopsis was the main disease found on young leaves and flower buds sampled on 27 May and on the branches and leaves of the 9 June samples. In samples of 8 July, Phomopsis stem cankers were fewer and milder in plants that were sprayed at 20% bloom only or at the V4 stage and 20% bloom compared to the controls.

Sclerotinia berry drop (a disease that was newly recognized in the course of the present work) was found on symptomatic leaves and flowers of the 9 June samples and in berries of 8 July samples. Lab tests showed that *Sclerotinia* was present in many berries that appeared healthy, but that the **incidence of *Sclerotinia* in the berries was reduced by 33 to 60%** (**or about half on average)** by the spray treatments (mainly the bloom spray). This reduction was especially good given that many flowers could not have been well protected by a single bloom spray (e.g. flowers that were not yet open or already fallen when bloom spray applied etc).

It should be noted that the diagnostic work on the diseases gave only approximate estimates of disease severity. Each of these diseases generally develops irregularly and in patches in the field.

CLONOSTACHYS SPRAY TREATMENTS DOUBLED THE PROPORTION OF HIGH QUALITY BERRIES AT HARVEST

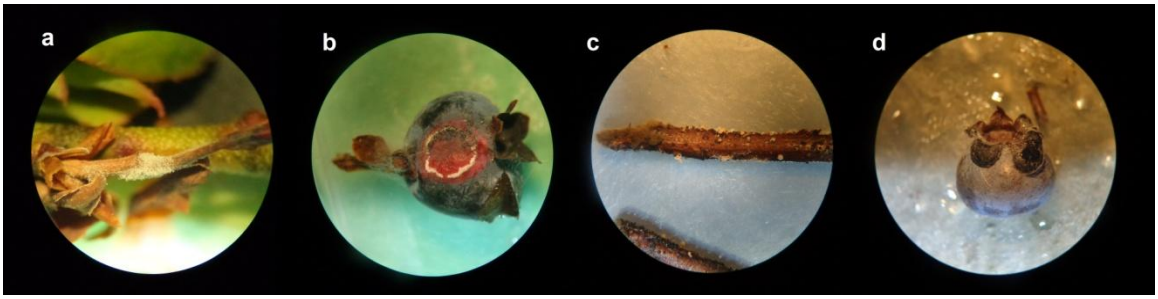
A single spray of *Clonostachys* at 20% bloom increased the proportion of harvested berries that were of good quality (spherical and firm) by 2.2 fold at site #1 and 1.3 fold at site #2. Two sprays at the V4 stage combined with 20% bloom increased the proportion of good quality berries by 2.3 fold at site #1 and 1.9 fold at site #2. The poor quality berries were generally split or collapsed, which may have resulted from mummyberry and Phomopsis infection. The results suggest that the bloom spray alone was effective for maintaining good quality in a high *proportion* of berries. However, as indicated above, two applications generally gave a much higher total berry yield.

SOME PRINCIPAL CONCLUSIONS REGARDING THE TIMING OF *CLONOSTACHYS* SPRAYS

- A spray application of *Clonostachys* a few days before bloom (V4 stage) combined with a second spray at 20% bloom resulted in substantially heightened berry yields (46% to 116%) and in the quality of the berries at harvest. These two applications also strongly reduced the severity of mummyberry, Phomopsis canker and Sclerotinia berry drop. It is considered that the suppression of these diseases by *Clonostachys* was a major factor in the huge yield increases found in the trials.
- A single spray at 20% bloom increased berry yield by 69% in one trial. While the bloom spray markedly reduced severity of Sclerotinia, a spray at the V4 stage combined with the bloom spray provided more effective control of mummyberry and Phomopsis than did the bloom spray alone.

CLONOSTACHYS SPRAYS ARE HIGHLY COST EFFECTIVE

Clonostachys spray treatments cost less than current fungicide treatments. No fungicide is currently registered for controlling Phomopsis or Sclerotinia in blueberries in Canada.



Monilinia sporulation on stem tissue (a), Monilinia conidia on dead berry (b), masses of Phomopsis spores exuding from a cankered blueberry stem (c), and small black sclerotia of Sclerotinia on dead berry (d).

***John Sutton** is a plant health consultant based near Ariss, Ontario. He served as professor of plant pathology at the University of Guelph (1969-2004) and later as adjunct professor (2005-2012).
** **Bradford Rooney** is a field biologist with PEI Berries Ltd in Montague, PEI.
***Todd Mason** is an agricultural consultant based in Oakville, Ontario.

Acknowledgements: This work was supported by PEI Berries Ltd of Montague, Prince Edward Island in a project funded by the Industrial Research Assistance Program (IRAP) of the National Research Council (NRC) of Canada. Funding was also provided by the PEI Wild Blueberry Growers Association. We are also pleased to acknowledge collaboration of the University of Prince Edward Island.